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Validation of mouse traumatic brain injury model with behavioral and MRI end-points

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BACKGROUND

Traumatic brain injury (TBI) is a leading cause of mortality and survivors of TBI frequently experience long-term disabling changes in cognition and sensory-motor function. Animal models have been developed to replicate the various aspects of human TBI to better understand the underlying pathophysiology and to explore potential treatments. The aim of the current study was to validate cortical contusion injury in mice with behavior assays for functional recovery and T2-MRI imaging. Validation of mouse model will also allow later combination of TBI with transgenic animals and creation of double hit models for TBI research.

MATERIALS AND METHODS

Animals

All animal experiments were carried out according to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals, and approved by the National Animal Experiment Board, Finland. 20 Male C57BL/6J mice were purchased from Charles River, Germany and weighing 20-25 g were used for the experiment. Animals were housed at a standard temperature (22 ± 1 °C) and in a light-controlled environment (lights on from 7 am to 8 pm) with *ad libitum* access to food and water.

TBI Procedure

In order to generate TBI, a computer controlled cortical PinPoint impact was carried out according to Bilgen M. with modifications (Bilgen M, Neurorehabil Neural Repair, 2005). The mice were anesthetized with isoflurane in 70% N_2O and 30% O_2; flow 300 ml/min. 2-3 min anesthesia induction with 5% isoflurane and maintenance with 1-2% isoflurane thereafter. The rectal temperature was maintained at 37.0 °C ± 1.5 °C with a homeothermic blanket system. Cortical contusion was induced using a PinPoint device (PintPoint™ Precision Cortical Impactor™, Hatteras Instruments, Inc). Each animal was placed in a stereotactic frame and a unilateral craniectomy of 3 mm in diameter was performed 2 mm posterior to bregma and 2 mm lateral to midline. The dura was kept intact and impact was done with a 2,5-mm-diameter tip traveling at a velocity of 3.0 m/s and creating a 2 mm-deep deformation. The brain surface was hit only once with the impactor tip (no repeated hits) and the impactor tip was in the brain for 80 ms. Thereafter the wound was closed with sutures and mice were allowed to recover in homeothermic cages before returning to the home cage. Abnormally excessive bleeding after contusion injury, clear indication of failed contusion (outside of craniectomy site epicenter) or other surgical complications were considered to be exclusion criteria in the study.

MRI

MRI acquisition was performed in a horizontal 7T magnet with bore size 160 mm equipped with a gradient set capable of max. gradient strength 750 mT/m and interfaced to a Bruker Avance III console (Bruker Biospin GmbH, Ettlingen, Germany). A volume coil (Bruker Biospin GmbH, Ettlingen, Germany) was used for transmission and a two-element surface array coil for receiving (Rapid Biomedical GmbH, Rimpar, Germany). Isoflurane-anesthetized mice (70% N_2O and 30% O_2; flow 300 ml/min, induction with 5%, maintenance 1.5%) were fixed to a head holder and positioned in the magnet bore in a standard orientation relative to gradient coils. Lesion size, tissue viability (T2 in milliseconds) and brain edema is determined using absolute T2 MRI. Multi-slice multi-echo sequence was used with following parameters; TR = 2.5 s, 12 different echo times (10-120 ms in 10 ms steps) and 4 averages. Eighteen (18) coronal slices of thickness 0.7 mm were acquired using field-of-view 20x20 mm² and 256x128 imaging matrix.

Behavioral testing

Body curl – Contralateral torso flexion is normally a component of multi-component point-based neurological examinations. Here it was used as a stand-alone test, during which mice were hand-suspended by the tail by an investigator and rated for degree of torso flexion from vertical towards the contralateral injury side. A numerical scoring system is based on the degree of contralateral body curl: absent (1), mild (2), moderate (3), and severe (4). Normal mice hang vertically without flexion and thus deviate 0° from vertical (rating of 1). Flexion of the torso 22.5° from vertical is rated a 2 (mild); flexion between 22.5° and 45° from vertical is rated 3 (moderate); and flexion of the torso 45° or more and with/or without grasping of the hindlimbs by forelimbs is given a rating of 4 (severe).

Rotarod – One day session included a training trial of 5 min at 4 RPM on the rotarod apparatus (AccuScan Instruments, Columbus, USA). One hour later, the animals were tested for 3 consecutive accelerating trials of 6 min with the speed changing from 0 to 40 RPM over 360 seconds and an inter-trial interval at least 30 min. The latency to fall from the rod was recorded.

Adhesive tape removal test – the test was used to assess sensorimotor deficits. Animal was placed in a see-through plexiglass box for 60 seconds habituation before test was started. Adhesive tape was applied on each forepaw of the animal the time-to-contact and the time-to-remove were measured. Three test trials were conducted and animals were followed for a maximum time of 120 with each round.

RESULTS

MRI

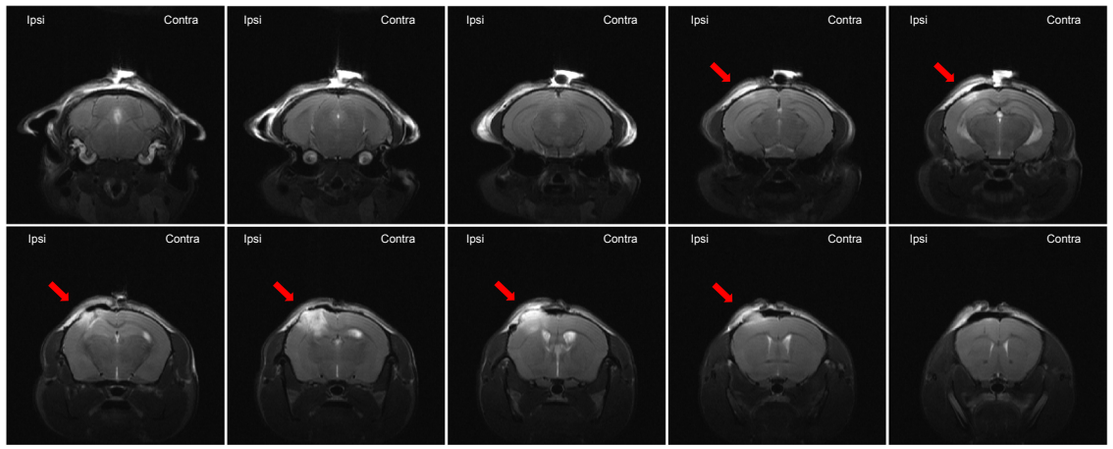

Figure 1. Representative MRI images at 24h post TBI. Coronal slices of 0.7 mm thickness were acquired using field-of-view 20x20 mm² and 256x128 imaging matrix.

BEHAVIOR

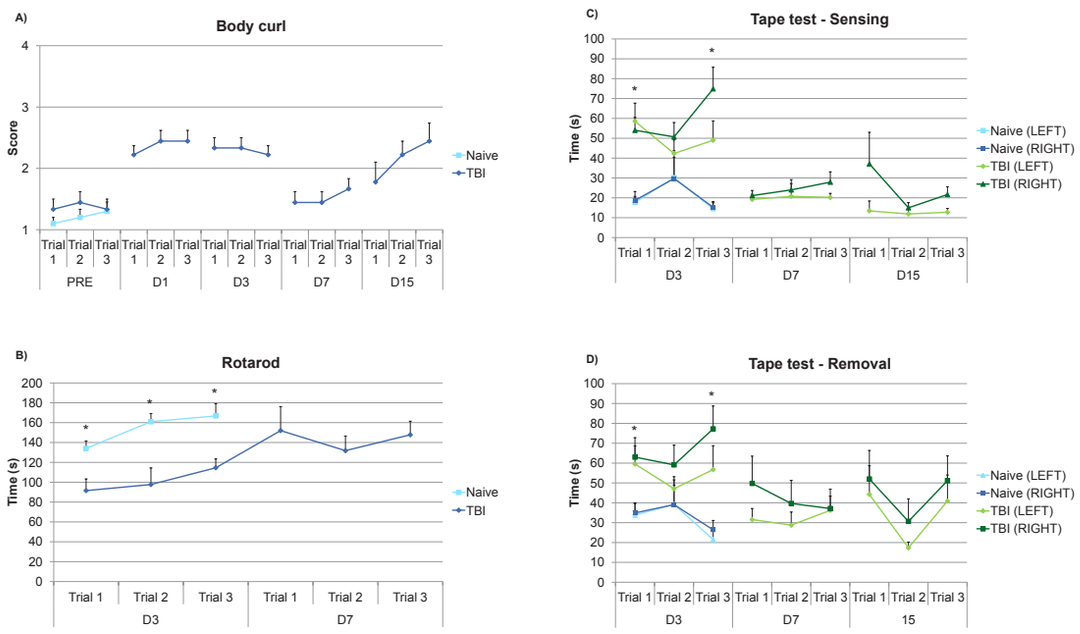

Figure 2. Behavioral data on body curl, rotarod and tape test. Body curl (A) and rotarod (B) showed a clear defect on days 1-3 and recovery on day 7. Tape removal test showed a clear deficit on day 3 on both removal and sensing (C) and recovery (D) on days 7 to 15. Presented as mean ± SEM. Statistical significances: *p < 0.05, Naive vs. TBI (Student t-test).

RESULTS

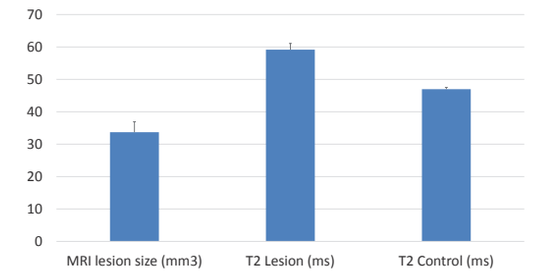

Figure 3. MRI data on lesion volume (mm³) and tissue viability presented as T2 relaxation time (ms) for the lesion and control side of the brain. Presented as mean ± SEM.

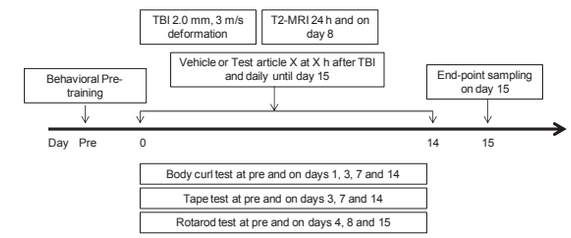

Figure 4. Suggested Study Schematic for TBI Studies.

Figure 5. Surgery lab at CR Discovery Services Finland

CONCLUSIONS

- The mice with cortical contusion injury exhibited clear functional deficits and later functional recovery in the behavioral assays for sensory-motor performance.
- Non-invasive imaging with T2-MRI provided information on the lesion size and tissue viability showing clear lesions as well as increased T2-relaxation time.
- The validated mouse TBI model offers a tool to study TBI in mice allowing combination with various transgenic lines and making it a valuable model for TBI research and drug discovery.
- Validation of mouse model will also allow later combination of TBI with transgenic animals and creation of double hit models for TBI research.